Remarks/Arguments

Submitted herewith are copies of the patents and literature references discussed and distinguished on pages 1 to 4 of the instant application and Patent No. 4,103,001 to Schattner. It is pointed out that the Buchalter (U.S. 3,983,252) and Bruckner et al. (U.S. 4,851,449) patents distinguished are now used by the Examiner in combination with the Hoover (U.S. 3,562,157) patent to reject the claims under 35 U.S.C. 103(a).

Also, it is to be noted that of the patents submitted, Pepper et al. (U.S. 3,016,328), Stonhill (U.S. 3,282,775), and Bruckner et al. (U.S. 4,971,999), were issued with product and process claims showing that examining claims to both product and process in this art does not present an undue burden to the Office.

On this point of restriction, the Examiner now states "whether or not bleach is a high level disinfecting composition in not an issue." In fact, it is THE issue. The original restriction requirement of July 1, 2005 stated as one basis for restriction that the process for using the product as claimed can be practiced with another materially different product and set forth bleach was such a product. Applicants responded by pointing out the method claims require a high level disinfectant and that bleach in not a high level disinfectant. Thus, that basis for supporting restriction was untenable and for the Examiner to now state that this is not an issue is disingenuous. The process claims require a conjugated aliphatic dialdehyde for high level disinfection and the fact that bleach is not a conjugated aliphatic dialdehyde and cannot provide high level disinfection is very relevant on the issue and restriction.

The submission of patents examined by the Office having claims to both product and process in the same field shows that a search for both does not present an undue burden to the Office. Thus, both bases for restriction set forth by the Examiner are in error.

The amendments to the Specification have been submitted to correct an inadvertent error made on page 4 in the Amendment of December 9, 2005. Therein it was requested that the paragraph on page 5, lines 14-22 be replaced with the amended paragraph set forth. This was an inadvertent error and the correct page and line should have been "page 6, lines 11-14." Also, "32" has now been substituted for "24" in line 19 of page 5, to conform to claim 10. This is not a new issue. The necessary amendment to page 5, lines 14-22 to correct the misspelling of "aliphatic" had already been made on page 3 of the December 9, 2005 Amendment.

Also, the misspelling of "sequestrant" on page 7, line 14 has been corrected and inclusion of "odor suppressant" made to conform to the specification on page 6, line 13 and original claims 6 and 14. This is not a new issue.

The specification on page 6, line 13, and independent claims have also been amended to recite that the aliphatic dialdehyde has less than 8 carbon atoms and at least one aldehyde group adjacent to a double bond. This was set forth in the claims 4 and 12 as originally filed and thus is not a new issue.

Also, claims 6 and 14 have been amended to correct the misspellings of suppressants and sequestrants and to substitute the word "or" for – and – to make them conform with the disclosure on page 7, line 14. This is also not a new issue.

This leaves for discussion the rejection of claims 1-6 and 10-14 under 35 .S.C. 103(a) as being unpatentable over Hoover, of record, in view of Buchalter (U.S. 3,983,252) and Bruckner et al., also of record. The defects of Hoover have been previously pointed out by Applicants in their Amendment of December 9, 2005. Not only has the Examiner failed to address these but has failed to show that it would be obvious to one skilled in the art to combine the secondary references with Hoover. Nothing is set forth in the Final Rejection explaining how the secondary references are to be combined with Hoover and why it would be obvious to one skilled in the art to do so.

It is therefore requested that the Finality of the rejection be withdrawn and that the Examiner set forth the missing explanation and permit Applicants the opportunity to respond thereto.

Having nothing concrete to respond to, Applicants can only state, based on the references themselves, that the combination of references would not be obvious to one skilled in the art and that even if combined, the references fail to disclose or suggest Applicants' claimed inventions.

It is again pointed out that Hoover does not disclose or suggest a sterilant/high level disinfectant and the use of a buffering agent and requires the use of hydroquinone, a known toxic material. While hydroquinone might be sensible for use in secondary oil recovery, it could not be used in medical environments where Applicants' products and those of the secondary references are to be used. Also, hyroquinone is not a buffering agent.

It is to be noted that Hoover may have a composition which is initially at a pH of 7 or below, but it is not buffered to maintain the pH at the desired level during storage and use. See lines 6 to 12 on page 7 of Applicants' specification.

Nor would it be obvious to eliminate the hydroquinone or to add a buffer thereto. Further, as noted in Applicants' previous Amendment, the amount of malealdehyde used in Hoover is at most 20 ppm (0.0002% by weight), compared to the range of 0.5% to 1% by weight required by Applicants' composition to be an effective sterilant/high level disinfecting product.

As to the secondary references, they do not disclose or suggest the use of a conjugated aliphatic dialdehyde having less than 8 carbon atoms and at least one aldehyde group adjacent to a double bond. Buchalter requires the use of a saturated aliphatic dialdehyde and Bruckner et al. an aromatic dialdehyde. In this art these are important differences and this is highlighted by the fact that the Buchalter patent was cited against Bruckner et al. and the Bruckner et al. patent allowed thereover showing that it was not obvious to those skilled in this art to substitute the aldehyde of one for the other. It is even more unobvious to substitute the malealdehyde of Hoover since Hoover, for the reasons discussed above, is not directed to a high level sterilant/disinfecting composition.

Further, the Examiner is in error by stating it "would have been reasonably expected" that the unsaturated aliphatic dialdehyde would be as similarly effective against bacteria as the saturated aliphatic dialdehyde of Buchalter. This is nothing but an "obvious to try" rejection. That is an impermissible standard for a rejection under 35 U.S.C. 103, which section of the statute requires a showing that it would have been obvious to produce the

instant claimed invention without the benefit of hindsight. See *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.* 1 USPQ2d 1081 (Fed. Cir. 1986)

See also *In re Sernaker* 217 USPQ 1 (Fed. Cir. 1983) wherein the Court stated at p. 6:

> "prior art references in combination do not make an invention obvious unless something in the prior art references would suggest the advantage from combining their teachings." (emphasis added)

In this case the prior art references show nothing to suggest that their combination as suggested by the Examiner would give the advantages of the Applicants' claimed invention.

The combination attempted is based solely on impermissible hindsight using Applicants' disclosure as a guide and taking bits and pieces from each of the cited references to arrive at Applicants' claimed invention without any suggestion in the art for such combination.

The references cited on page 1 through 4 of the application, two of which are already of record, have been submitted herewith solely to show the state of the art and that the Patent Office allowed:

(1) Pepper et al. Pat. No. 3,016,328 to glutaraldehyde as sterilizing agents;

(2) The Stonehill Pat. No. 3,282,775 to saturated dialdehydes as sterilizing agents over Pepper et al. which was cited by the Examiner during it prosecution;

(3) Boucher et al. Pat. No. 3,708,263 limited to a method and apparatus for continuously sterilizing medical equipment utilizing compositions comprising glutaraldehyde and was allowed over the Pepper et al. and Stonehill patents cited during its prosecution;

(4) Boucher Pat. No. 3,912,450 to the use of glutaraldehyde as a sterilizing composition was allowed over the Pepper et al. and Stonehill patents cited during its prosecution;

(5) Boucher Pat. No. 3,968,248 to glutaraldehyde as a sterilizing solution over the Pepper et al. and Stonehill patents sited during its prosecution;

(6) Boucher Pat. No. 3,968,250 to glutaraldehyde as a sterilizing agent over Pepper et al. and Stonehill cited during its prosecution;

(7) Buchalter Pat. No. 3,983,252, already of record in this case, directed to a saturated dialdehyde as a high level sterilizing and disinfectant solution was allowed over Pepper et al. and Stonehill cited during prosecution;

(8) Schattner Pat. No. 4,103,001 to the use of glutaraldehyde as a sterilizing composition was allowed over the Stonehill and the four above-noted Boucher patents cited during prosecution;

(9) Jacobs Pat. No. 4,436,754 to the use of glutaraldehyde as a sterilizing composition was allowed over the Pepper et al., Stonehill, two of the Boucher patents noted above, Buchalter, and Shattner patents cited during its prosecution;

(10) Bruckner et al. Pat. No. 4,847,304 to the use of a glutaraldehyde combined with an aromatic dialdehyde as a sterilizing composition was allowed over the Pepper et al., Stonehill, four Boucher patents noted above, Buchalter, and Jacobs patents cited during its prosecution;

(11) Buckner et al. Pat. No. 4,851,449, already of record in this case, to the use of a disinfecting and sterilizing composition was allowed over the Pepper et al., Stonehill, the four Boucher, Buchalter, and Jacobs patents cited during its prosecution;

(12) Bruckner et al. Pat. No. 4,971,999 to the use of phthalaldehyde as a sterilizing and disinfecting solution was allowed over three of the four Boucher, Jacobs, and Bruckner et al. 4,851,449 patents cited during its prosecution; and

(13) The Gorden et al. article shows efforts to enhance the mycobacterial activity of glutaraldehyde by using therewith certain unsaturated and aromatic aldehydes which posses little, if any, activity on their own.

All the foregoing patents and Gorden et al. article are directed to sterilizing and disinfecting agents to be used to sterilize medical equipment and the problems in this field, as is Applicants' invention. Not one discloses or suggests the use of a conjugated aliphatic dialdehyde as claimed by Applicants. Moreover, Applicants show on page 3 of their specification that the glutaraldehyde compositions disclosed in the above-noted patents once deemed effective are now less effective as determined by the newest method referred to as the Quantitative Tuberculocidal Test Method.

In contrast, the Hoover patent is to a completely unrelated field, secondary oil recovery, does not use buffering agents, and uses a toxic additive, hydroquinone, making it unsuitable for medical use. To modify the Hoover patent for use in the medical field by eliminating hydroquinone and adding buffering, surfactants, and other ingredients to enhance the efficacy, stability and materials compatibility of the products shown in the above-cited patents would not be obvious to one skilled in this art, but simply as exercise in hindsight particularly since there is no patent that shows or suggests that malealdehyde can function as a high level sterilant and disinfectant. It alone cannot do so any more than bleach can. The fact that a compound can kill certain bacteria does not mean it will be effective as a high

level disinfectant that will also destroy spores and meet the standards of the noted new test. Applicants have made a patentable advance in the art showing how a conjugated aliphatic dialdehyde can be used to form high level sterilizing and disinfecting agents, a clear and patentable advance in the art.

For the forgoing reasons, reconsideration is required of the restriction requirement and rejection of the claims and the allowance of all the claims is respectfully requested.

References discussed above and submitted herewith include: (1) Pepper et al. Pat. No. 3,016,328; (2) Stonehill Pat. No. 3,282,775; (3) Boucher et al. Pat. No. 3,708,263; (4) Boucher Pat. No. 3,912,450; (5) Boucher Pat. No. 3,968,248; (6) Boucher Pat. No. 3,968,250; (7) Buchalter Pat. No. 3,983,252; (8) Schattner Pat. No. 4,103,001; (9) Jacobs Pat. No. 4,436,754; (10) Bruckner et al. Pat. No. 4,847,304; (11) Buckner et al. Pat. No. 4,851,449; (12) Bruckner et al. Pat. No. 4,971,999; and (13) The Gorden et al. article

Dated: May 8, 2006

Respectfully submitted,

Robert M. Mason, Reg. No. 33,067
402 Carillon Tower West
13601 Preston Road
Dallas, Texas 75240
Telephone: (972) 788-1500
Facsimile: (972) 788-1561

ATTORNEY FOR APPLICANT

CERTIFICATE OF MAILING

I hereby certify that the above-noted paper was deposited with the United States Postal Service first class mail, postage prepaid in an envelope addressed to: Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, sent on May 8, 2006.

Robert M. Mason

Journal of Industrial Microbiology, 13 (1994) 77–82

Published by The Macmillan Press Ltd

Enhancement of mycobactericidal activity of glutaraldehyde with α,β-unsaturated and aromatic aldehydes

M. D. Gordon, R. J. Ezzell, N. I. Bruckner and J. M. Ascenzi

Research Division, Johnson and Johnson Medical, Inc., PO Box 130, Arlington, TX 76004, USA

(Received 28 May 1993; revision received 2 October 1993; accepted 21 October 1993)

Key words: Glutaraldehyde; Mycobactericidal; Aldehydes

SUMMARY

Several α,β-unsaturated and aromatic aldehydes were evaluated for antimicrobial activity using *Mycobacterium bovis* as the test strain. Activity of most of the compounds was determined in the presence and absence of 2% glutaraldehyde. Several compounds highly active against this organism, e.g. 2-pentenal, benzaldehyde, and *o*-phthalaldehyde showed rapid kill of $>10^5$ CFU ml^{-1} in 5 min. Activity of α,β-unsaturated compounds substituted in the β, position showed increasing activity with increasing chain length. Of the aromatic aldehydes tested, benzaldehyde and *p*-dimethylamino benzaldehyde showed little activity alone, but when combined with 2% glutaraldehyde showed increased activity. Substituents added to the benzaldehyde ring (nitro, chloro, methyl, and methoxy) all detracted from the synergism, but still showed increased activity over the activity of 2% glutaraldehyde. The same affect was noted with disubstituted benzaldehyde compounds but not with substituted *o*-phthalaldehyde (2-formylformaldehyde).

INTRODUCTION

Aldehydes have biological activity which includes inhibition of metabolism in both eucaryotic [28,29] and procaryotic organisms [7,14,20,22,23], antitumor effects [30], cell division [12], and static and cidal activity against various bacteria [2,4,26], fungi [1,10,11,13], and viruses [3,18,27]. Several aldehydes occur naturally and have antimicrobial activities that provide protective mechanisms. For example; *trans*-2-hexenal, a fungicide, occurs naturally in the damaged leaves of *Gingko biloba* protecting the leaves from infection by fungi [19]. Citral and other terpene aldehydes found in grasses inhibit the growth of microbes in the stomachs of ruminants, thereby hindering complete utilization of ingested food [24].

Several factors influence the activity of aldehydes, such as carbon chain length, substituent groups, and degree of bond saturation. These factors affect the reactivity of the carbonyl carbon with sulfhydryl and amino groups, thereby affecting reaction with proteins and nucleic acids. Damage or rearrangement of these molecules can be the cause of the effects mentioned above. α,β-Unsaturated aldehydes react primarily with sulfydryl groups (primarily on proteins) forming a saturated aldehyde with a thioether linkage. The reactivity of the carbonyl group of the α,β-unsaturated aldehyde is affected by substituent groups on the carbon chain. The activity of aromatic aldehydes is also related to the reactivity of the carbonyl carbon(s), as demonstrated by the fact that while benzaldehyde is an active antimicrobial, benzyl alcohol and benzoic acid are not. The reactivity is also affected by substituent groups on the aromatic ring [28].

Glutaraldehyde (GA) is one of the most biologically active alkyl aldehydes in terms of its ability to kill bacteria, fungi, and viruses [5,16]; however, it does not possess the rapid mycobactericidal activity required by users of liquid disinfectants [9]. Extended exposure time above that effective for other vegetative cells is required for complete kill of $\geq 10^5$ CFU *Mycobacterium bovis* BCG. This is true for *Mycobacterium tuberculosis*, and most of the atypical mycobacteria. It has been demonstrated with *Mycobacterium cheloniae*, that a barrier to aqueous solutions exists [17] and lack of penetration of aqueous antimicrobials into the cell may account for their reduced activity.

In this paper we report the mycobactericidal activity of α,β-unsaturated aldehydes and aromatic aldehydes for their activity in the presence and absence of GA. Mycobactericidal activity is examined as a measure of activity since mycobacteria appear to be the most difficult of the vegetative organisms to inactivate with GA. Any increased activity provided by the added compounds can easily be discerned.

MATERIALS AND METHODS

Mycobacterial assays

Mycobacterium bovis ATCC 35743 was grown according to a previously published method [2]. Cells were stored in 2-ml aliquots at −70 °C until used. Frozen cultures were thawed at room temperature, diluted with saline containing 0.05% Tween 80 (Difco, Detroit, MI, USA) to a titer of approximately 10^5 CFU ml^{-1}. One milliliter of the cell suspension was added to 9 ml of the test solution at 20 °C

Correspondence to: J.M. Ascenzi, Research Division, Johnson and Johnson Medical, Inc., PO Box 130, Arlington, TX 76004, USA.

and aliquots removed at time intervals, diluted in an eqnal volume of neutralizer, serially diluted in saline and collected on 0.45-μm pore size membrane filters (Millipore Corp., Medford, MA, USA) which were placed onto Middlebrook 7H10 (Difco) with OADC (oleic acid-albumin-dextrose-catalase) enrichment (Difco) agar plates. Duplicate samples were plated and incubated for 21 days at 37 °C and counted using a binocular microscope. Data were plotted as survivors (S/S_0, where S is the number of organisms at any given time point and S_0 is the number of organisms at zero time).

Test solutions

GA solutions were prepared at a 2% concentration at a pH of 7.5. α,β-Unsaturated and aromatic aldehydes were added to the GA at the same molar concentration or to the limit of their solubility.

Neutralizer

Neutralization of aldehydes was accomplished with sodium bisulfite at a concentration 2.2 times the concentration of the total aldehyde concentration in the test solution. Antimicrobial activity of the neutralizer was assessed by the addition of equal volumes of neutralizer and test solution and spiking the mixture with 10^6 CFU ml^{-1} *M. bovis* and incubating it under the test conditions and plating for survivors up to an hour.

RESULTS

α,β-Unsaturated aldehydes

The structures of the compounds studied are shown in Table 1. Table 2 shows the relative activities of α,β-unsaturated monoaldehydes when combined with 2% GA solution. 2-Propenal had the greatest enhancement of activity against *M. bovis* BCG. This compound has no substituents on the carbon backbone. All other substituted compounds, except 2-hexenal, showed less activity, but still showed enhanced activity over 2% GA. Substitution in both β-positions (3-methyl-2-butenal) or in the α- and one of the β-positions (2-methyl-2-butenal), resulted in reduced activity relative to that of 2-propenal. 2-Hexenal and 3-phenyl-2-propenal are two compounds in this series that had low

TABLE 1

Structure of α,β-unsaturated aldehydes studied

2-propenal	$H_2C{=}CH{-}CHO$
3-methyl-2-butenal	$H_3C{-}C(CH_3){=}CH{-}CHO$
2-methyl-2-butenal	$H_3C{-}CH{=}C(CH_3){-}CHO$
trans-2-hexenal	$H_3C{-}CH_2{-}CH_2{-}CH{=}CH{-}CHO$
2-pentenal	$H_3C{-}CH_2{-}CH{=}CH{-}CHO$
2-butenal	$H_3C{-}CH{=}CH{-}CHO$
3-phenyl-2-propenal	$C_6H_5{-}CH{=}CH{-}CHO$
2,4-hexadienal	$H_3C{-}CH{=}CH{-}CH{=}CH{-}CHO$

TABLE 2

Effect of α,β-unsaturated monoaldehydes on mycobactericidal activity of alkaline 2% glutaraldehyde solutions[a]

2% Glutaraldehyde + monoaldehyde (% w/w[b])	Time for total kill at 20 °C (min)
None (2.0% GA)	>90
2-propenal (0.19)	5–10
2-methyl-2-propenal (0.23)	30
2-butenal (0.23)	30
2-methyl-2-butenal (0.28)	60–70
3-methyl-2-butenal (0.28)	40–45
2-pentenal (0.28)	30
2-hexenal (0.33)	10
3-phenyl-2-propenal (0.10[c])	20
2,4-hexadienal (0.32)	20

[a]Test solutions were buffered with 0.6% dipotassium hydrogen phosphate. The pH of the test solutions was adjusted to 8.0 with 1N H_3PO_4.

[b]Equivalent to 3.33 mmol of monoaldehyde per 100 g of solution, except as noted.

[c]Solubility limit, equivalent to 0.77 mmol per 100 g of solution.

toxicity (as indicated by their widespread use in the food/flavor industry) and good activity enhancement. The most active compound, 2-propenal, is a highly toxic compound not likely to be used in products having the potential to come into contact with humans.

Of the series of 2-alkenal compounds in which an alkyl group is substituted in one β-position, the activity enhancement follows the order:

2-Hexenal > 2-Pentenal > 2-Butenal

β_1 = $CH_3CH_2CH_2$ CH_3CH_2 CH_3

The longer the alkyl chain, the greater is the activity (substituents greater than four carbons are not water soluble). The activity of several of the α,β-unsaturated monoaldehydes in the presence and absence of 2% GA is shown in Fig. 1. The data indicate enhanced activity of the combination over the activity of the 2% GA or monoaldehyde alone. 2-Butenal and *trans*-2-hexenal showed activity at low concentrations (<0.5%). 2-Propenal (0.19%) achieved complete kill of 10^5 CFU ml^{-1} *M. bovis* in ≤2 min. Neutralization controls indicate complete neutralization of the antimicrobial activity.

Aromatic aldehydes

A series of studies was done looking at the effect of mycobactericidal activity of aromatic aldehydes (structures shown in Table 3) in combination with GA. Table 4 shows increased activity with the addition of 0.3% benzaldehyde (BA) to solutions of 0.5 to 2.0% GA. Complete kill of >10^5 CFU ml^{-1} was achieved in <20 min even with the lowest level of glutaraldehyde tested, whereas 2% and 3% GA alone require >60 min for complete kill of the same test population.

Several monosubstituted BA compounds (Table 5) were evaluated in combination with 2% glutaraldehyde. Substitu-

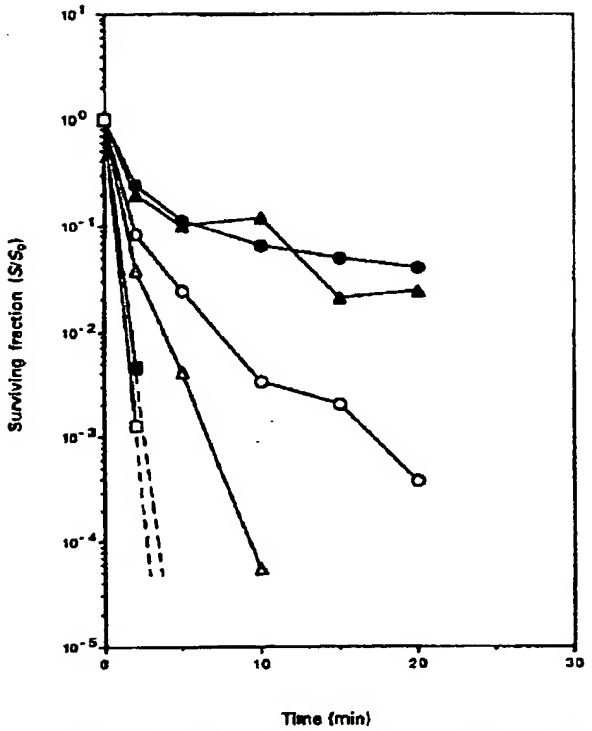


Fig. 1. Mycobactericidal activity of 2-butenal (—●—), *trans*-2-hexenal (—▲—), glutaraldehyde (—○—), GA + 2-butenal (—△—), GA + *trans*-2-hexenal (—□—), GA + 2-propenal (—■—). 2-propenal alone produced complete kill in <2 min.

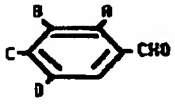
ents may also be present in the 2 (ortho-) and 3 (meta-) positions of BA with retention of good mycobactericidal activity enhancement. Although none had the enhancement effect of benzaldehyde, all provided enhancement that increased the activity over that of 2% glutaraldehyde. The activity of two representative aromatic aldehydes, benzaldehyde and *p*-dimethylamino benzaldehyde in the presence and absence of 2% GA are shown in Fig. 2. Neither showed activity alone, however, each increased the mycobactericidal activity of 2% GA.

The disubstituted BA compounds, 4-hydroxy-3-methoxy-, 3-hydroxy-4-methoxy-, 3,4-dioxymethylene-, 3,4,-dihydroxy-, and 3,4-dimethoxy-, were evaluated for activity when added to 2% GA at concentrations ≤0.55%. All resulted in enhancement activity and 3,4-dioxymethylene benzaldehyde had the greatest affect (Table 6). The aromatic dialdehyde, 2-formylbenzaldehyde (*o*-phthalaldehyde, OPA) was evaluated for activity when added to 2% GA. The activity of this combination was due largely to OPA as shown in Fig 3. These data indicate that OPA rapidly inactivates 10^5 CFU ml^{-1} *M. bovis* in less than 10 min at all three concentrations tested with complete kill in less than 5 min at 20 °C with 0.45% OPA.

Several monosubstituted OPA compounds (4-methoxy-, 4-hydroxy-, 4-chloro-, and 4-carboxy-OPA) were compared to OPA for mycobactericidal activity in the absence of 2% GA (data not shown). The activity of 0.13% 4-methoxy-OPA was somewhat greater than that of an equimolar solution of OPA, while the 4-chloro compound had similar activity. However, the 4-hydroxy- derivative had less activity than OPA, and 4-carboxy-OPA had no substantial activity as did the trisubstituted OPA compound 3-carboxy-4-methoxy-5-methyl-OPA.

TABLE 3

Structure of aromatic aldehydes evaluated



Compound	Substituent groups		
	A	*B*	*C*
Benzaldehyde	H	H	H
2-Hydroxybenzaldehyde	OH	H	H
3-Hydroxybenzaldehyde	H	OH	H
4-Hydroxybenzaldehyde	H	H	OH
4-Methoxybenzaldehyde	H	H	OCH_3
4-Methylbenzaldehyde	H	H	CH_3
4-Chlorobenzaldehyde	H	H	Cl
4-Nitrobenzaldehyde	H	H	NO_2
p-Dimethylaminobenzaldehyde	H	H	$N(CH_3)_2$
4-Hydroxy-3-Methoxybenzaldehyde	H	OCH_3	OH
3-Hydroxy-4-Methoxybenzaldehyde	H	OH	OCH_3
3,4-Dioxymethylenebenzaldehyde	H	OCH_3	OCH_3
3,4-Dihydroxybenzaldehyde	H	OH	OH
3,4-Dimethoxybenzaldehyde	H	OCH_3	OCH_3
2-Formylbenzaldehyde	CHO	H	H
4-Methoxy-2-Formylbenzaldehyde	CHO	H	OCH_3
4-Hydroxy-2-Formylbenzaldehyde	CHO	H	OH
4-Chloro-2-Formylbenzaldehyde	CHO	H	Cl
4-Carboxy-2-Formylbenzaldehyde	CHO	H	COOH
3-Carboxy-4-Methoxy-5-Methyl-2-Formylbenzaldehyde	CHO	COOH	OCH_3 (D-CH_3)

TABLE 4

Effect of glutaraldehyde concentration on mycobactericidal activity of benzaldehyde[a]

Concentration		Time for total kill at 20 °C (min)
Glutaraldehyde % (w/w)	Benzaldehyde % (w/w)	
2.0	—	>90
3.0	—	75
2.0	0.30	10
1.0	0.30	15
0.5	0.30	20

[a]See footnote a, Table 2.

OPA rapidly inactivates 10^5 CFU ml^{-1} *M. bovis* in less than 10 min at all three concentrations tested with complete kill in less than 5 min at 20 °C with 0.45% OPA.

TABLE 5

Effect of monosubstituted benzaldehydes on mycobactericidal activity of 2% alkaline glutaraldehyde solution[a]

Monoaldehyde (%w/w[b])	Time for total kill at 20 °C (min)
Benzaldehyde (0.30[c])	10
2-Hydroxybenzaldehyde (0.41) (salicylaldehyde)	20
3-Hydroxybenzaldehyde (0.41)	20
4-Hydroxybenzaldehyde (0.41)	30
4-Methoxybenzaldehyde (0.45) (*p*-Anisaldehyde)	20
4-Methylbenzaldehyde (0.18[d]) (*p*-Tolualdehyde)	20
4-Chlorobenzaldehyde (0.06[e])	20
4-Nitrobenzaldehyde (0.06[f])	30

[a]See footnote a, Table 2.
[b]See footnote b, Table 2.
[c]Solubility limit equivalent to 2.83 mmol per 100 g of solution.
[d]Solubility limit equivalent to 1.50 mmol per 100 g of solution.
[e]Solubility limit equivalent to 0.43 mmol per 100 g of solution.
[f]Solubility limit equivalent to 0.40 mmol per 100 g of solution.

TABLE 6

Effect of disubstituted benzaldehydes on mycobactericidal activity of 2% alkaline glutaraldehyde[a]

Monoaldehyde (%w/w[b])	Time for total kill at 20 °C (min)
Benzaldehyde (0.30[c])	10
4-Hydroxy-3-Methoxybenzaldehyde (0.51) (Vanillin)	30–35
3-Hydroxy-4-Methoxybenzaldehyde (0.20) (Isovanillin)	35–40
3,4-Dioxymethylenebenzaldehyde (0.30[c]) (Piperonal)	20
3,4-Dihydroxybenzaldehyde (0.46)	70–90
3,4-Dimethoxybenzaldehyde (0.55) (Veratraldehyde)	35

[a]See footnote a, Table 2.
[b]See footnote b, Table 2.
[c]See footnote c, Table 5.
[d]Solubility limit equivalent to 1.31 mmol per 100 g of solution.
[e]Solubility limit equivalent to 2.00 mmol per 100 g of solution.

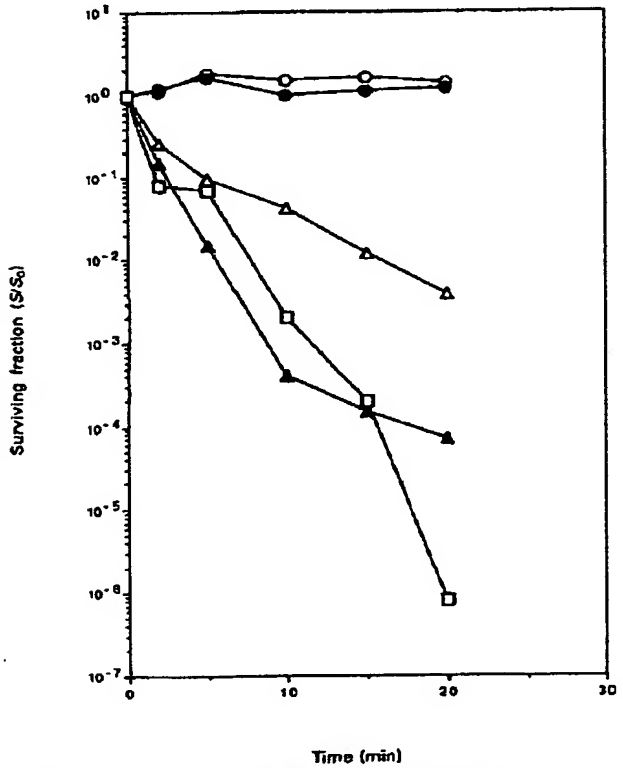


Fig. 2. Mycobactericidal activity of *p*-dimethyl amino benzaldehyde (—○—), benzaldehyde (—●—), GA (—△—), GA + *p*-DMB (—▲—), and GA + benzaldehyde (—□—).

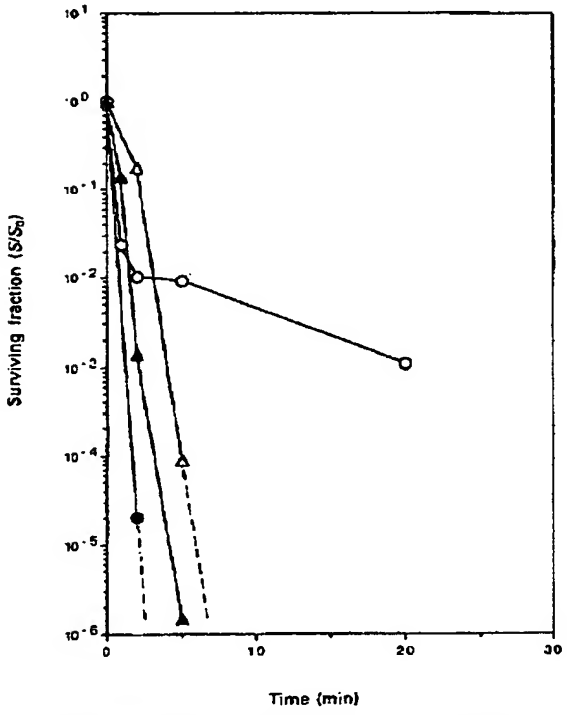


Fig. 3. Mycobactericidal activity of *o*-phthalaldehyde, 0.1% (—△—), 0.2% (—▲—), and 0.45% (—●—) compared to 2% glutaraldehyde (—○—).

DISCUSSION

Glutaraldehyde has been used in disinfectant formulations since the original description of the antimicrobial properties of GA by Pepper and Chandler [25]. The excellent biocidal properties have been documented regardless of the formulation although alkaline glutaraldehyde is a better sporicide than acid or neutral GA [15,21]. This pH dependence does not exist with other bacteria including mycobacteria.

The mycobactericidal properties of glutaraldehyde are less than its activity against other Gram-positive and Gram-negative bacteria. This is a concern for healthcare workers since GA has been the chemical of choice for disinfection of critical equipment. Cole et al. [8] showed that regardless of the claim on glutaraldehyde formulations, the reality of the situation requires that at least a 30-min soak of medical equipment is necessary for mycobactericidal activity whereas most vegetative organisms are eradicated in 10 min or less.

An alternative to GA as a disinfectant is desired because of the intermediate mycobactericidal activity, toxicity, and irritation potential. α,β-Unsaturated and aromatic aldehydes have been used in the flavor and food industry and therefore have been identified as safe for use in products that contact humans. An examination of their antimicrobial properties indicates that several may have excellent activity while others have none. In combination with GA, many of these additives can substantially increase the mycobactericidal activity of the formulation. Their activity appears to be related to the substituent groups on the molecule.

α,β-Unsaturated aldehydes show increased activity with increased chain length of the substituent group on the β_1 position, probably due to increased hydrophobicity and better penetrability of the molecule through the lipid matrix of the cell wall-membrane of mycobacteria. Disubstitution, whether in the α, β_1 or β_1, β_2 positions reduced the enhancement effect of the aldehyde significantly. This may be due to steric hindrance in the formation of the 1,4 addition product with amine or sulfhydryl groups, significantly reducing the antimicrobial activity of the aldehyde.

With the aromatic aldehydes, it is evident that either electron releasing (methoxy, methyl, chloro) or electron withdrawing (nitro) substituents in the 4 (para-) position of BA results in increased mycobactericidal activity. 4-Hydroxy BA showed the least amount of activity enhancement, which may reflect a pH dependency. Burton et al. [6] showed that the antimicrobial activity of substituted benzaldehydes was directly related to the partition coefficient of the aldehyde in hydrocarbon solvent. This indicated that activity may be related to its ability to penetrate the lipid cell wall-membrane complex. This is of particular relevance with the highly lipid cell wall-membrane complex of mycobacteria which possesses a barrier to aqueous solutes [17] lending support to the hypothesis that more hydrophobic chemicals are more likely to penetrate mycobacterial cells. Although penetration of the compound into the cell plays an important role in antimicrobial activity, the activity of the benzaldehyde compounds is directly related to the reactivity of the formyl group. While benzaldehyde is active at fairly low concentrations, benzoic acid and salicylic acid at concentrations as high as 10% in a 2% GA formulation show little or no activity enhancement over the activity of GA. This is supported by the work of Burton et al. [6] in which benzyl alcohol and benzoic acid were shown to be relatively inactive when compared to benzaldehyde.

REFERENCES

1 Aharoni, Y. and R. Barkai-Golan. 1973. Sensitivity to acetaldehyde vapours of *Alternaria tunuis* and *Stemphylium botryosum*. Phytopathol. Zeitschrift 78: 57–61.

2 Ascenzi, J.M., R.J. Ezzell and T.M. Wendt. 1987. A more accurate method for measurement of mycobactericidal activity of disinfectants. Appl. Eviron. Microbiol. 53: 2189–2192.

3 Bachrach, U., S. Don and H. Wiener. 1971. Antivirus action of acrolein, glutaraldehyde and oxidized spermine. J. Gen. Virol. 13: 415–422.

4 Beilfuss, W. 1976. Vergleichende untersuchungen der antimikrobiellen wirksamkeit α,β-ungesattigter aldehyde. Zbl. Bakt. Hyg., I. Abt. Orig. A 234: 271–280.

5 Boucher, R.M.G. 1975. On biocidal mechanisms in the aldehyde series. Can. J. Pharm. Sci. 10: 1–7.

6 Burton, D.E., K. Clarke and G.W. Gray. 1964. The mechanism of the antibacterial action of phenols and salicylaldehydes. Part III. Substituted benzaldehydes. J. Chem. Soc. July (1964): 2458–2460.

7 Chan, K. and T.M. Lau. 1979. Effect of formaldehyde on oxygen uptake and β-galactosidase activity in *Enterobacter uerogenes*. Microbios. Lett. 10: 69–74.

8 Cole, E.C., W.A. Rutala, L. Nessen, N.S. Wannamaker and D.J. Weber. 1990. Effect of methodology, dilution, and exposure time on the tuberculocidal activity of glutaraldehyde-based disinfectants. Appl. Environ. Microbiol. 56: 1813–1817.

9 Collins, F. 1986. Comparison of bactericidal activity of alkaline glutaraldehyde solution against a number of atypical mycobacterial species. J. Appl. Bacteriol. 61: 247–251.

10 Dabrowa, N., J.W. Landau and V.D. Newcomer. 1972. Antifungal activity of glutaraldehyde in vitro. Arch. Dermatol. 105: 555–557.

11 Deanis, C. and A. Gaunt. 1974. Effect of formaldehyde on fungi from broiler houses. J. Appl. Bacteriol. 37: 595–601.

12 Egyud, L.G. 1967. Studies on cell division: the effect of aldehydes, ketones and α-keto-aldehydes on the proliferation of *Escherichia coli*. Curr. Molec. Biol. 1: 14–20.

13 Gorman, S.P. and Scott, E.M. 1977a. A quantitative evaluation of the antifungal properties of glutaraldehyde. J. Appl. Bacteriol. 43: 83–89.

14 Gorman, S.P. and E.M. Scott. 1977b. Transport capacity, alkaline phosphatase activity and protein content of glutaraldehyde-treated cell forms of *Escherichia coli*. Microbios. 19: 205–212.

15 Gorman, S.P. and E.M. Scott. 1977c. Effect of alkalinization of the bacterial cell and glutaraldehyde molecule. Microbios. Lett. 6: 39–44.

16 Gorman, S.P., E.M. Scott and A.D. Russell. 1980. A review. Antimicrobial activity, uses and mechanism of action of glutaraldehyde. J. Appl. Bacteriol. 48: 161–190.

17 Jarlier, V. and H. Nikaido. 1990. Permeability barrier to hydrophilic solutes in *Mycobacterium chelonei*. J. Bacteriol. 172: 1418–1423.

18 Kremzner, T.L. and D.H. Harter. 1970. Antiviral activity of

oxidized polyamines and aldehydes. Biochem. Pharmacol. 19: 2531–2550.

19 Major. R.T., P. Marchini and A.J. Boulton. 1963. Observation on the production of α-hexenal by leaves of certain plants. J. Biol. Chem. 238: 1813.

20 McGucken, P.V. and W. Woodside. 1973. Studies on the mode of action of glutaraldehyde on *Escherichia coli*. J. Appl. Bacteriol. 36: 419–426.

21 Munton, T. J. and A.D. Russell. 1970. Aspects of the action of glutaraldehyde on *Escherichia coli*. J. Appl. Bacteriol. 33: 410–419.

22 Munton, T.J. and A.D. Russell. 1972. Effect of glutaraldehyde on the outer layers of *Escherichia coli*. J. Appl. Bacteriol. 35: 193–199.

23 Munton, T.J. and A.D. Russell. 1973. Effect of glutaraldehyde on cell viability, triphenyltetrazolium reduction, oxygen uptake, and β-galactosidase activity in *Escherichia coli*. Appl. Microbiol. 26: 508–511.

24 Oh, H.K., T. Sakai, M.B. Jones and W.M. Longhurst. 1967. Effect of various essential oils from Douglas fir needles upon sheep and deer rumen microbial activity. Appl. Microbiol. 15: 777–784.

25 Pepper, R.E. and V.L. Chandler. 1963. Sporicidal activity of alkaline alcoholic saturated dialdehyde solutions. Appl. Microbiol. 11: 384–388.

26 Rehn, D. and H. Nolte. 1979. Zur antimikrobiellen wirksamkeit substituierter aromatischer aldehyde und alkohole. Zbl. Bakt. Hyg., 1. Abt. Orig. B 168: 507–516.

27 Sabel, F.L., A. Hellman and J.J. McDade. 1969. Glutaraldehyde inactivation of virus in tissue. Appl. Microbiol. 17: 645–646.

28 Scaife, J.F. 1970. Modification of the cytotoxic action of hydroxypentenal on cultured mammalian cells. Naturwissenschaften 57: 250–252.

29 Schauenstein, E. 1967. Autoxidation of polyunsaturated esters in water: chemical structure and biological activity of the products. J. Lipid Res. 8: 417–428.

30 Schauenstein, E., J. Zangger and M. Ratzenhofer. 1964. Uber die wirkung von hydroxyoctenal auf Ehrlich-acites-tumorzellen. Z. Naturforsch. 19b: 923.